

ECOTOX

ECOTOXicology Database System

AQUIRE Coding Guidelines

Prepared for

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REVIEW OF LITERATURE

A. OVERVIEW OF ECOTOX

1. Introduction to Review of Literature

The data elements included in ECOTOX encompass standard test parameters typically reported within a publication. Each database record contains information about the exposure and test conditions. Specific parameters include the test chemical, species, and endpoint or effect concentration.

The included literature is identified through standardized bibliographic retrievals. Each publication is evaluated and the applicable data is encoded by trained literature reviewers. The data encoded are evaluated according to existing standard test methods such as those from the American Society for Testing and Materials (1996), Code of Federal Regulations (1992), and the American Public Health Association (1992). Each test included in ECOTOX is assigned a documentation code that indicates the amount of supporting methods and results documentation available in the original scientific publication.

Note: Each publication included into the AQUIRE database must meet the five minimal criteria for acceptance (i.e. chemical, species, concentration, duration and effect) . If the paper is missing one or more of these criteria ECOTOX does not search other sources to obtain the missing data piece(s). Sources such as author communications and referencing another work to obtain one of the five criteria is allowed for specific risk assessment/criteria projects (e.g. EcoSSL or CAD) and must be specified by the EPA Database Coordinator.

2. Literature Reviewer Training

Training Sequence

The training sequence is designed to develop consistent, accurate, and versatile literature reviewers. This is accomplished through an intensive period of literature review, interactive quality assurance procedures, and consultation with other ECOTOX database personnel.

The scope of the six month intensive training period encompasses the following areas:

- endpoint toxicity test review (one month);
- effect only toxicity test review (two months);
- bioconcentration study review (one month);
- field study review (one month); and
- in-depth training within the areas listed above (one month).

The personnel available to support the reviewer include the data coordinator and trained ECOTOX reviewers. The following documentation and materials are used for training:

- ECOTOX Standard Operating Procedures (2001, www.epa.gov/ecotox under “download files”);
- Fundamentals of Aquatic Toxicology (Rand (Ed) 1995);
- American Society for Testing and Methods; (ASTM, 1996);
- Selected toxicity literature publications; and
- AQUIRE and TERRETOX coding sheets.

The reviewer initially reviews the ECOTOX Standard Operating Procedures: Coding Guidelines, applicable publications listed in the reference section for each of the databases, applicable US EPA Standard Evaluation Procedures and ASTM guidelines. The primary emphasis is to understand the minimum criteria that characterize acceptable toxicity tests. These criteria must be reported in the toxicity publications selected for review in order to qualify for inclusion in the ECOTOX database. The acceptance criteria are:

- Name of the test **chemical**;
- Name of the **test organism**;
- **Effect** of the test chemical on the organism;
- Test chemical **concentration** or application rate;
- Test **duration** (except for abstracts and non-English publications).

The secondary emphasis is to develop the ability to distinguish between exposure types (lethal, sublethal, bioconcentration). The reviewer is trained to recognize whether standard methods are reported for test methodologies and for the test endpoint. The reviewer is also trained to identify tests which are not applicable to ECOTOX.

Once the general introductory materials are read, the standard training guidelines introduce the reviewer to each category of toxicity literature. Information specific to areas of acute, chronic and bioconcentration literature is discussed in subsequent sections of this chapter. The guidelines can be tailored to the specific areas of expertise and strengths that each person brings to the project. Three primary elements are emphasized in each component of the training sequence. The standard training sequence is:

1. Example review: Examination of previously encoded toxicity literature. The trainee reviews between 5 to 10 toxicity publications and compares each with its associated pre-completed coding sheet.
2. Independent review: The trainee independently reviews a minimum of 10 to 20 toxicity publications. All 10 - 20 reviews are quality assured via a review of the publication and coding by the data coordinator. Inconsistent coding practices are resolved with the trainee. The trainee continues to review additional toxicity publications and the level of QA decreases from 100 percent to 10 percent as the reviewer's consistency and proficiency increase.

3. Measure of proficiency: Established ECOTOX quality assurance procedures require a close review of all reviewed publications by the data coordinator to ensure accurate reviewing is consistent with current test methodologies and SOPs. All discrepancies identified are noted by the data coordinator and discussed with the trainee.
4. A full time reviewer begins the training sequence reviewing 20 publications per month. This amount increases until a level of 35 publications per month is attained. The average time estimated per review at the beginning of the training sequence is 1.5 hours per publication. The time should decrease to one hour per publication. A part time reviewer's training expectations will be decreased accordingly.

Measures of Competency for Trained Reviewers

The quality assurance process is an ongoing component of literature reviewing. Emphasis is placed on quality assurance during the initial collaborative training period, during the 10 percent replicate review process, and through consultation with publications in the field of aquatic toxicology. As part of this process, consistency and concurrence between the document abstractors is attained.

The ten percent replicate review process assures data integrity and promotes routine evaluation of coding practices. Through this training process, strengths and weaknesses in the data abstractor's expertise are identified and specific programs are established to enhance expertise where needed. Such programs include consultation with ECOTOX staff, toxicology publications and the EPA Database Manager, as needed. Evaluation of replicate reviews, which is performed on 10% of all coded references, is used to flag and correct any major discrepancies between replicates. In addition a screening of all completed coding sheets to ensure consistency and completeness prior to data entry is required. Parameters routinely screened include water chemistry, test organism descriptors, calculated endpoints and total test numbers.

Steps in the Quality Assurance Process

1. Ten percent of the reviewed articles from each abstractor are randomly identified by the data coordinator. Information concerning the number of publications is entered into a Lotus 1-2-3 file, maintained on the data coordinator's computer. The spreadsheet tracks the QA process and calculates the percent of the publications subjected to quality assurance for each reviewer (Table 1). The original reviewer's code sheet for the chosen publication is placed in the "Double Review and QA" file folder maintained by the data coordinator. An "ECOTOX 10% Tracking Form" sheet is maintained in the folder and filled out as articles are received (Attachment 1.). The spreadsheet file is also updated.

Table 1. ECOTOX 10% TRACKING SHEET EXAMPLE

Date Rec	Doc #	Tot Rec'd	# QAed	2nd Rev	2nd Comp	Coord	Completed
11/30/93	5342	8	1	JACKY	11/30/93	ANNE	01/15/94
12/14/93	6808	10	1	AMY			

2. The publication is given to a second reviewer for independent review. After completion of the second review, the data coordinator gives the coding sheets and paper to the EPA Database Manager who compares both reviewer's coding sheets, documents the differences (if any) between reviewers, archives the information on the "ECOTOX 10% Replicate Review" form (Attachment 1.), then returns the form to the reviewers for comment. The reviewers note discrepancies by either agreeing with the EPA Database Manager's comments or expressing their differing opinions on the form. After the replicate review form is returned to the EPA Database Manager, discussions are held with both reviewers to resolve any remaining differences. Discrepancies due to differences in interpretation are resolved by the EPA Database Manager. Errors caused by incomplete Coding Guideline documentation are identified and modifications are made to the document.
3. Upon completion of the review process, the data coordinator checks to make sure the original reviewer's coding sheet contains the correct data, notes completion date on the "ECOTOX 10% Tracking Form" and in the spreadsheet, and forwards the coding sheet to data entry. The ECOTOX 10% Replicate Review forms are filed with the double review coding sheets in the ECOTOX Reviewed files.

3. General Coding Information

Overview

ECOTOX is comprised of two databases - AQUIRE and TERRETOX. The AQUIRE database is comprised entirely of aquatic data whereas the TERRETOX database is comprised of terrestrial data. These databases were developed independently but are merging together under the ECOTOX framework. Across the two databases, the common data elements for each test contained in ECOTOX are grouped by chemical, organism, exposure conditions, and effects. Test chemical parameters describe the toxicant and any associated carrier; the CAS registry number; and the grade, purity and/or composition. The test organism parameters include the Scientific name, a species number and lifestage, source, and/or characteristics of the organism. The test conditions identify the test location; exposure type, time, and conditions; and any control parameters. Effect and endpoint parameters consist of codes to define lethal, sublethal, or residue effects and/or endpoints. The corresponding chemical concentration or dose is reported for both exposure and observation

concentrations, if reported. Available data are extracted from the text, tables, and graphs of each publication.

Based on the information coded for the preceding categories, a documentation code is calculated for each piece of data in ECOTOX. The documentation code provides an index of the completeness of methods documentation and results presentation in the original publication.

The following sections are designed as an overview of the guidelines for reviewers. The information presented in this section identifies the common and unique attributes of each database. Each section heading corresponds to a data element (if the data element is unique to one or two of the databases, this is noted following the section heading). The unique attributes of each database are described in the specific coding guidelines for AQUIRE and for TERRETOX. Any exceptions from these guidelines must be authorized by the EPA Database Manager and subsequently documented in these guidelines.

Coding Practices

This section provides an overview of the general coding practices used for the ECOTOX database. These practices have been devised to ensure accuracy and consistency in transcribing data from the original publication to the final data file.

- A unique coding sheet is used for each of the independent databases - AQUIRE and TERRETOX.
- Each reported test exposure (or in some cases each unique endpoint) requires a separate line on the coding sheet. If many tests are reported that are conducted under similar conditions, ditto marks are placed in the field or remarks area where the information is identical to the line above.
- Endpoints, effect or exposure concentrations/doses, control data and exposure times reported in graphic format are coded.

Data extracted from graphs are presented as range or <, > values, unless an exact value is clearly presented. If the format of the graph does not allow extrapolation, the availability of such data is noted in REMARK, i.e., "/control data graphed/". Data extracted from a graph must be accompanied by a comment in the REMARK field "/from graph/". When there is a discrepancy between data presented in the text or table and data presented in a graph, the paper is to be forwarded to the EPA Database Manager for a final determination of which data point will be included in the database.

- To ensure completeness and accuracy, if information is unavailable for a coding field, the field must still be completed using either NR (not reported) or occasionally, NA (not applicable).

- To ensure accuracy in transcribing data values, all numbers between zero and one should be reported with a zero preceding the decimal point (e.g., 0.5 not .5). Periods are only used to represent a decimal point, never an abbreviation.
- To ensure consistency as well as accuracy, report the significant figures as the author reports them. Do not add or round off numbers. Report only the actual values, do not code variance information (e.g. +/-).
- When coding numbers do not use commas. They can be mistaken for decimal points or numbers.
- Use “per” or a colon (:) instead of a slash (/) to designate ratios. Reserve the slash for designating remarks or units.
- The REMARK field is a text field which contains additional information about a coding field. The REMARK field is used when the information necessary for coding a field does not fit in the space provided. A complete list of remark identifiers is documented in the appendices for each of the databases.
- When making a remark, use the appropriate codes from the ECOTOX code list. If a code does not exist for a certain chemical, enzyme, device, technique, etc., do not use the author’s abbreviation. Write out the full name of the term and submit to the ECOTOX staff to determine if a new code should be created.
- All coding sheets with the same reference number and the same chemical CAS number are stapled together in the upper left corner.
- Coding sheets are generally double sided. If any tests are coded on the back of the coding sheet, the “continued on back” text located in the lower right corner of the coding sheet is highlighted in yellow.
- When a reviewer has completed the review of a paper, they must write their last name preceded with “R=” on the bottom center of the paper.

B. AQUIRE CODING GUIDELINES

A unique coding sheet is used for each of the independent databases - copies of the AQUIRE coding sheets are located at the end of this section. For AQUIRE, field (natural and artificial) tests are coded on the AQUIRE Field Coding Sheet; all other studies are coded on the AQUIRE Lab Coding Sheet.

1. Quality Assurance Parameters

QA Date/Initials

The person conducting the first Quality Assurance Check enters the date of the QA check and their initials.

Publication Reference Number, Author, Year

The Reference Number (Ref #) is the unique number which identifies a particular publication. This number, assigned by the data entry program, provides the link between the data entered and the original publication. On the coding sheet, enter the reference number located in the upper right-hand corner of the hard copy of the publication, the last name of the first author, and the publication year. For abstracts, use the publication year of the abstract source.

Total Tests

The total tests encoded for a publication are recorded by the reviewer. The total test number equals the total number of individual effect records that are coded for each publication.

Reviewer/Date

The reviewer's last name is written here. The date on which the publication was reviewed should be entered in the format of month/day/year.

2. Test Chemical Parameters

AQUIRE is catalogued by the toxicant tested using the Chemical Abstracts Service (CAS) registry number. If a CAS registry number is not available through standard sources the toxicity data cannot be included in AQUIRE. Additional toxicants not included in AQUIRE are water chemistry effects (e.g., pH), complex effluents, and chemical mixtures.

Chemical mixtures may be interpreted broadly. For example, if a pesticide is a mixture of two active ingredients, each may have a separate CAS number. If the formulation has a CAS number, the chemical reported for AQUIRE is the formulation. If the exposure is based on two metal compounds but the effect is based on one ion, e.g., copper sulfate and copper chloride and Cu is the toxicant, code copper as the test chemical and report the two compounds in chemical CHARACTERISTICS.

For *in situ* exposures where the exposure is by default an exposure to a chemical mixture; code residue effects or endpoints (BCF) only. No other effects or endpoints are strictly attributable to a single chemical in the same way as a residue concentration. Data for chemicals in the mixture with reported water concentrations and residue effects should be coded. The only situation in which a mixture exposure can be coded is when an *in situ* field study is conducted. The test organisms must be transplanted from a clean source and caged in the polluted source. The duration and concentration must be provided. When the author provides only the species age for the duration, it is unacceptable to code.

Effect responses from exposures to hormones (e.g. estradiol, testosterone) are included in the database. There are studies where the hormone is administered as a toxicant to observe the andro/estrogenic effects.

Studies involving carbon dioxide (CO₂) or ozone (O₃) as the toxicant are not coded into the AQUIRE database.

Nutrients such as phosphorus, nitrogen, potassium are coded for AQUIRE if the exposure system is dosed rather than an ambient exposure. For example, code phosphorus as an exposure chemical if, in the given paper, all of the following are true:

- The phosphorus was added to the ecosystem in a direct discrete manner, i.e., code *"nylon mesh bags of Ca(H₂PO₄)₂ placed in streams at beginning of test"*, do not code *"system may have received added phosphorus in overland runoff due to fertilizers used in nearby agricultural operations"*. Aerial applications are acceptable if the other conditions are met.
- The concentration in the water should be measured, or at a minimum, the application rate should be available. Application rate may be calculated using the flow volume and the phosphorus-containing compound's dissolution rate.
- The effects of the phosphorus are tested on a biological test organism; water quality or chemical-fate only papers are not coded.

Effect responses from exposures to humic acid only are not coded for AQUIRE. Humic acids are any various complex organic acids obtained from humus which are insoluble in acids and organic solvents. However, tests that include an exposure with a toxicant and humic acid should not be interpreted as a mixture. The humic acid information should be coded into the ORGANIC CARBON field if the concentration is given and "Humic Acid Efcts" should be coded in the OTHER EFFECTS field.

Example: The toxicant is Copper and Humic acid is added at 10 mg/l.
Code the concentration of Humic acid in the ORGANIC CARBON field as 10 mg/L HA.

Chemical Name (TEST)

Record the chemical name as it is reported in the publication; however, long chemical formulas or names need not be coded if a common name is provided. For common names, record common name in both the TEST field and the CHARACTERISTICS field; when the CAS number is entered into the system the 9CI Preferred Name will be assigned automatically. The TEST field on the coding sheet is used for the convenience of the encoder in assigning the CAS number. If several names (e.g., trade names, synonyms) are used, note the other names and formula in parenthesis after the recorded chemical name.

The CAS number is assigned by locating the chemical name in the chemical card file or in the online index file (ECOCHEM). If the chemical name is not in the chemical card file or ECOCHEM, write "No" near the CAS NUMBER field to clearly identify that verification is needed. The coding sheet will be referred to ECOTOX staff for CAS number verification as part of the quality assurance process.

Chemical Grade (GRADE)

Record relevant chemical grade information in the GRADE field. (refer to ECOTOX Appendix B).

Chemical Purity (PURITY)

Record the numeric percentage information about the purity or active ingredient of the chemical in the PURITY data field (e.g., if the author reports 97% purity, 97 would be entered into this data field. PU for purity would be entered into the FORM data field (see CHEMICAL FORMULATION).

Chemical Formulation (FORM)

Record the chemical formulation code for the chemical reported. If there is more than one formulation code enter the code most closely related to the chemical purity, and enter the rest in the CHARACTERISTICS field. (refer to ECOTOX Appendix C)

Chemical Comments (CHARACTERISTICS)

Record relevant and specific chemical information, such as trade names, common names, isomers, or extra formulation codes, but do not code lot numbers or product numbers (usually taken from chemical catalogs). There are times when you will record the chemical name in both the TEST field and the CHARACTERISTICS field. This occurs most frequently for pesticides where the common or trade name is very simple while the chemical nomenclature is very complex. The purpose, during reviewing, for the name in TEST field is to assist the reviewer in assigning a CAS number; during data entry the name is replaced by a stored 9CI Preferred Name. The common name coded in CHARACTERISTICS remains available for user access.

Radiolabel (RADIOLABEL)

If a radiolabeled chemical is tested, record the isotope, according to the ECOTOX Appendix D

codes, in the RADIOLABEL field. When the specific isotope is not reported, the field should be coded with a slash ("/") and noted in the REMARK field (RADIO/no isotope reported/). When both radiolabeled and unlabelled test chemicals are used in a test, report the radiolabel isotope and code "labelled and unlabelled" in CHARACTERISTICS.

CAS Number (CAS NUMBER)

The Chemical Abstracts Service Registry Number of the toxicant is recorded in the CAS NUMBER field. A standardized identification number and name for each chemical recorded in the database is used for consistency. Toxicants included in the ECOTOX database are assigned a CAS registry number and are referred to by the Ninth Collective Index (9CI) standard nomenclature. The CAS number and 9CI name are stored in a chemical card file and in an online index file (ECOCHEM) which is available electronically for screening CAS numbers and chemical names used in ECOTOX. If a hydrated form of a chemical is used in the paper, record the hydrated form as reported by the author in the TEST field. However, record the CAS Number for the non-hydrated form of the chemical in the CAS NUMBER field.

Example:	Chemical cited in paper	CuSO ₄ * 5H ₂ O	CAS# 7758998
	<u>TEST</u> field	CuSO ₄ * 5H ₂ O	
	<u>CAS NUMBER</u> field	CAS# 7758987 (CuSO ₄)	

Solvent Chemical (S/V)

If a solvent carrier is used in the test, the solvent chemical fields are coded with the Solvent Chemical (S/V), GRADE, PURITY, FORM, concentration (in CHARACTERISTICS) and CAS NUMBER. The CAS numbers for common carriers are listed in ECOTOX Appendix A.

Occasionally two or more separate carriers or solvents are used. If the publication reports the ratio, include this information in the CHARACTERISTICS field. If the carrier or solvent is used for different test chemicals but the use is not specifically described in the publication, code "as needed" in the CHARACTERISTICS field.

If a carrier was not used, report as NR. Buffers used to control the pH of the test are not coded. Acids or bases that are added to change the pH of a solution in order to enable a metal to stay in solution are not coded. Dietary feed content is not coded.

Occasionally, an author will present results data related to effect of the solvent on the test organism. If a test concentration and results are reported for the solvent and there is a separate clean water control, the solvent data should be coded on a separate coding sheet.

If an author states that all solvent was evaporated prior to the study or if a column coating procedure is described, the solvent is assumed to not be incorporated into the study and should be coded as NR in the S/V field.

For instances where a chemical carrier does not have a CAS number and it is determined by the chemical verification staff that it will never have a CAS number, the solvent will be entered in the CHEMICAL COMMENTS field of the Chemical Name (TEST).

If the solvent does not have a CAS number and it is in the verification process, data entry will enter NR in the S/V field and enter a remark of CARRIER/solvent name and characteristics//. When the CAS# is verified a search is made in the remarks for the chemical name and those records found are modified.

Example: The solvent used is Atlox at 0.05 ul/l. There is no CAS# and it is in the verification process.
Entered into REMARKS: CARRIER/ATLOX, 0.05 UL/L//

If an author presents results in which the test organism is exposed to multiple chemicals, it is important to determine if the two chemicals constitute a mixture or if one of the chemicals is being used as a carrier or solvent. A carrier is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with the dilution water before distribution to test chambers (Rand, 1995). If the other chemical is not a carrier, "mixture" would be noted in OTHER EFCT.

Water should not be coded as a solvent. A solvent is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with dilution water before distribution to test chambers. Solvents or carriers are used in toxicity tests where the concentrations of the test chemical are extremely low and a very small amount of test material must be added to the test chambers. (Rand, 1995)

3. Test Organism Parameters

Species (LATIN NAME/SPECIES NUMBER)

Record the species Scientific name as reported in the publication. A unique number is assigned to each ECOTOX species to aid in storage and retrieval. Reviewers locate the number for each test organism from the CRITTERS species file and record this number on the coding sheet. If the species name reported in the publication is a synonym of a verified species, record the name from the publication, draw a line through it and record the verified species name along with the species number. If the species is not on the verified name list, write "No" near the Scientific name to clearly identify that verification is needed. The coding sheet will be referred to ECOTOX staff for species verification as part of the quality assurance process. Refer to Species Procedures for additional information about the species data file and verification procedures.

Field studies may report results for a target community (e.g. benthic macroinvertebrates) or for an entire enclosed ecosystem (e.g. system-level primary productivity or respiration). If a community of organisms was tested, be as specific as the author is about the species grouping.

Organism Comments (ORGANISM CHARACTERISTICS)

Report any general information provided about the initial condition of the test organism. This includes information for both the control and test organisms. Organism comments include information such as age, weight, length, developmental stage, sex, type of culture (eg., axenic) and/or initial cell concentration (e.g. 1 E + 3 cells/ml or expo gro phase or log gro phase) to describe the organism being tested. Each piece of information is separated by a comma. The value and range, if reported, are recorded for each available parameter (e.g. 3 (2-4) g). However, deviations are not coded (e.g. 33 +- 4 mm is coded as 33 mm). Record strains, hybrids or taxonomic groupings, if reported. List individual species Scientific names when 3 or fewer species are included within a grouping; when more than 3 individual species are included within a grouping, code as "# species".

Species = Plankton (#706)

ORG CHARACTERISTICS = *Daphnia magna*, *Daphnia pulex*, and *Bosmina sp*
ORG CHARACTERISTICS = 4 zooplankton species

Standard terms used for recording organism length include standard length (SL), (e.g. 3.1 cm SL), total length (TL), fork length (FL), carapace length (CL), carapace width (CW), wet weight (wetwt), and dry weight (dry wt).

Tests in which eggs are initially exposed, and the exposure continues through adulthood to the first generation etc, are represented as "Egg" in ORGANISM CHARACTERISTICS and the stage of the organism is recorded in the EE REMARK field for the results reported.

Example:

Exposed eggs resulting in mortality of fry

ORG CHARACTERISTICS: Egg

EFCT: MOR

MEASURE: MORT

EE REMARK: Fry

If the paper states that the organisms tested are both male and female, this characteristic does **not** go into the ORGANISM CHARACTERISTICS field, because a sample assumes both sexes. However, if only one sex is tested, then the sex is coded using the terms "male" or "female".

If a paper reports results using organisms from a polluted source AND organisms from a non-polluted source, only the non-polluted source test results are coded. No mention of 'non-polluted' needs to be presented in the ORGANISM CHARACTERISTICS field, but 'polluted organisms' should be entered into OTHER EFCT data field. If data are presented for organisms from a polluted source and no other concurrent data with organisms from a clean source are presented, the reviewer should code the test results, but enter 'polluted organism' in the ORGANISM CHARACTERISTICS data field.

If a paper reports that the organisms used for testing were diseased, "Diseased Organisms" is entered into the EXPERIMENTAL DESIGN field. If the study was conducted on diseased organisms compared to non-diseased organisms, the data from the non-diseased organism test is coded, but the data from the diseased organisms is not. In this case, "Diseased Organism Test" is entered into the OTHER EFCT field.

If a paper reports that the organisms used for the measurements were dead and it is unknown how long the organisms have been dead, do not code this data, but enter "dead organisms" in OTHER EFCT.

If the study was conducted on dead organisms compared to living organisms, the data from the living organism is coded but the data from the dead organism test is not coded. In this case, dead organism is entered into the OTHER EFCT field.

If some of the organisms tested are fed differing amounts and/or some of the organisms are not fed during the study and the authors are comparing the data (e.g. 10 organisms fed, 10 organisms not fed and/or some organisms fed 10 pellets and some organisms fed 5 pellets), all data are coded, "Fed", "Not Fed" or food amount is coded in the EXP DESIGN field, and "Feeding Efcts" is coded in the OTHER EFCT field.

The source of the organism does not need to be coded in the ORG CHARAC field unless the author(s) compare results based on the source of the organisms. "Organism source efcts" should also be noted in the OTH EFCT field.

Example: ORG CHARAC: Hatchery-reared
 OTH EFCT: Organism Source efcts//

Control Type (CNTL)

The type of test control(s) used in the study is reported in this field. Control information for the reported effect may be presented in the text, in a graph, or in table format. ECOTOX reviewers do not make assessments whether the controls were satisfactory or insufficient (e.g., were replicates run, did control organisms die), but simply document whether the author(s) present information that a control was used. When author's state that controls were similar to treatment with the exception that no chemical was added, and within the same paragraph they describe using solvent in all treatments, a solvent control should be interpreted. (refer to ECOTOX Appendix M for control type codes and definitions.)

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), code M in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in EE REMARK. (e.g., stats based on solvent control//) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such in the EE REMARK field.

When multiple controls are reported by the author, code "M" in the CONTROL TYPE field and list the various controls reported in a remark.

Example: Author reports the use of clean water control and a solvent control

CODE: CONTROL TYPE: M/ REMARK: CONTR/C,V//

4. Test Condition Parameters

Media Type (FW, SW)

The type of water or media is coded in the FW,SW field. Freshwater (FW) tests include 1) laboratory tests conducted in freshwater, reconstituted water, distilled water, or tap water or 2) field tests where the organism habitat is exclusively freshwater. If a salinity value of <4 ppt is reported and the paper does not specify whether it is fresh or saltwater, it will be coded as a freshwater test.

Saltwater (SW) tests include 1) laboratory tests conducted in natural or artificial seawater, brackish water, or estuarine water or 2) field tests where the organism habitat is exclusively saline.

If a determination cannot be made regarding the use of either freshwater or saltwater, an NR (not reported) is recorded.

Test Location (LAB, NR, FIELDN, FIELDA, FIELDU)

Report the location or setting in which the experiment was conducted (see ECOTOX Appendix H).

For example, a natural field study (FieldN) is an experiment conducted outdoors in a natural setting in which the test organisms are confined via an enclosure of some type (cage, fencing, plot lines) or sampled in the wild. Field exposures are exposed to uncontrolled variables such as weather. An important component for classification as natural is that the setting includes a bottom substrate as well as a community of representative organisms. Outdoor studies conducted in a simulated environment are coded as an artificial field study (Field A) study. Such studies include organisms isolated from their natural environment while still out of doors, e.g. earthen or concrete ponds without sediment or with only one representative species.

Laboratory tests are conducted under indoor controlled laboratory conditions (light and temperature regulated). If the location or setting cannot be determined from the publication code as Not Reported (NR). For AQUIRE, field (natural and artificial) tests are coded on the AQUIRE Field Coding Sheet; all other studies including tests not specified field or lab, are coded on the AQUIRE Lab Coding Sheet.

Study Type (STUDY TYPE)

For laboratory exposures, the study type is used to identify field simulation studies. For example, indoor mesocosm or microcosm studies should be noted as such in the STUDY TYPE field. If information about the study type is not reported, leave this field blank.

For field exposures record the study type as reported by the author in the STUDY TYPE field. Examples of field study types include, but are not limited to, exposures with caged organisms or conducted in a mesocosm, microcosm or enclosure. If information about the study type is not reported, leave this field blank.

Experimental Design (EXP DESIGN)

This field is used to code additional study information. For field tests, report exposure system dimensions (e.g. pond or lake depth, cage or enclosure size), type of artificial substrate and physical or chemical water chemistry parameters.

EXP DESIGN: 3 ha polyethylene lined pond//	EXP DESIGN: 4 x 4 m cage//
EXP DESIGN: sediment//	EXP DESIGN: humic acid//
EXP DESIGN: Instant Ocean®//	EXP DESIGN: Sinking Cr water//

For laboratory studies, information about media and test chambers is coded if one of the purposes of the study is to compare results observed under differing test conditions (e.g., pH, temp, humic acid, sediment) or if commercial media types (e.g. Instant Ocean®) were used in the study. If one of the purposes of the study is to compare experimental effects (pH, temp, sex) in addition to toxicant effects, report the additional effects in the OTHER EFCT field. (Refer to ECOTOX Appendix V for a list of keywords)

Example: OTHER EFCT: pH efct//

Information about the dilution water is provided if needed to distinguish one test scenario from another, e.g. natural waters from three different ponds, sites on a river, locations in a sea. Tests with differing dilution water are coded as separate lines of data; it is not acceptable to combine tests by effect or water chemistry variables across differing dilution water test scenarios.

If an organism is pre-exposed to another chemical and this is the only information that can be coded, the chemical that is associated with the observed effect is coded and “preexposure with X” is noted in the EXP DESIGN field.

On occasion, an author will note that organisms were sampled during the course of a study for analysis. “Sub-sampled” should be noted in the EXP DESIGN field.

Example: Author tests organisms for residue and mortality over a 30 day study. At day 5 and 10, several organisms are pulled from the study for residue analysis. At the end of the test (30d) the mortality is reported. “Sub-sampled” should be coded in EXP. DESIGN field since these sub-sampled organisms may or may not be included in the final calculation for mortality.

When coding field exposure publications, additional related coding parameters that do not get coded in the STUDY TYPE, HABITAT CODE, and SUBSTRATE fields are added to the EXPERIMENTAL DESIGN field. When adding field exposure information to the EXP DESIGN field, make sure to precede all data added with the appropriate field name code.

Example: EXP DESIGN: HAB/suspended mesh bags//

5. Test Result Parameters

Toxicity test results for the AQUIRE database are represented by a combination of the ENDPOINT,

TREND, EFFECT, RESP SITE, EE REMARK, MEASURE, EFCT%, SIG, LEVEL, CONC and BCF fields.

Toxicity test results for AQUIRE are primarily reported for observations taken during the chemical exposure; however, when results are reported *only* for the period of time after the exposure (moved to clean water), ie. recovery or delayed effects, this type of result is noted by using a “~” in conjunction with the endpoint/effect code, e.g. ~MOR for a delayed mortality effect.

Figure 1. shows the hierarchy of coding tests in AQUIRE.

Endpoints always require a discrete line. For data not reporting an endpoint, at least one separate line is coded for each measurement from either a unique experimental design or within one design scenario for statistically defined data points. .

Food chain effects or endpoints are coded for organisms at the first level of exposure. Subsequent levels of exposure are not coded, but are noted in the OTHER EFCT: field, e.g. Other Effects: food chain study//.

Endpoint, Effect, Measurement and Statistics sections have further description and examples.

The following sections provide a brief description for each of these fields, followed by guidance for coding information from the publication for each of the fields.

Endpoint (ENDPOINT)

For the purposes of AQUIRE, an endpoint is the quantification of an observed effect obtained through statistics or other means of calculation for the express purpose of comparing equivalent effects (e.g., LC50). ECOTOX Appendix T identifies and defines the ECOTOX endpoint codes. The endpoint field will be coded as NR if the author does not report or define an endpoint or there is no companion data point.

Endpoint information is coded into AQUIRE if it is reported by the author, if the author's definition of the effect is equal to AQUIRE endpoint definitions, or if the data point is a companion endpoint to a LOEC, NOEC and/or MATC. “Companion endpoints” are endpoints assigned by the reviewer when the statistical results follow a clear concentration-response pattern and the author reports a NOEC, LOEC or MATC but fails to report the “companion endpoint”. For example, when an author reports a NOEC and does not specifically define the lowest statistically significant effective concentration as a “LOEC”, the data point is coded as a LOEC in AQUIRE by the reviewer. Similarly for reported LOECs without NOECs, NOEC/ LOECs without MATCs and MATCs without NOEC/LOECs.

If within a study an author reports a NOEC and/or LOEC endpoint for one of the measurements in the text but does not specify the endpoints for the other measurements which show statistical data points within the same graph or table, the reviewer may extrapolate the SIG and NOSIG points for the other measurements as LOEC and NOEC using the R designation in the ASSIGNED ENDPT field. The reviewer may not extrapolate data from other tables or graphs. In studies where

measurements are reported with statistical significance but the author does not report a NOEC and/or LOEC for any of the measurements, the reviewer does not code a NOEC and/or LOEC.

Example: The text contains the following statement: "The NOEC and LOEC for weight were found to be 10 ug/l and 20 ug/l, respectively." A table reports weight data as well as length and mortality data which is statistically analyzed.

Conc (ug/l)	Length (cm)	Weight (mg)	Mortality (%)	* Sig at p<0.05
control	21	30	0	
5	22	30	5	
10	20	24*	5	
20	15*	20*	8	
30	13*	18*	25*	

Code:

Endpoint Assigned	Endpoint	Effect	Measurement	Conc	Signif	Level
P	NOEC	GRO	LGTH	10 ug/l	NOSIG	p<0.05
P	LOEC	GRO	LGTH	20 ug/l	SIG	p<0.05
R	NOEC	GRO	WGHT	5 ug/l	NOSIG	p<0.05
R	LOEC	GRO	WGHT	10 ug/l	SIG	p<0.05
R	NOEC	MOR	MORT	20 ug/l	NOSIG	p<0.05
R	LOEC	MOR	MORT	30 ug/l	SIG	p<0.05

On occasion, authors will report LC50 information in the methods section of their publication, without reporting any accompanying test procedure information. These test results are coded if enough information is provided by the authors to verify that the value(s) were not published elsewhere, and that the study meets all five minimal criteria for acceptance.

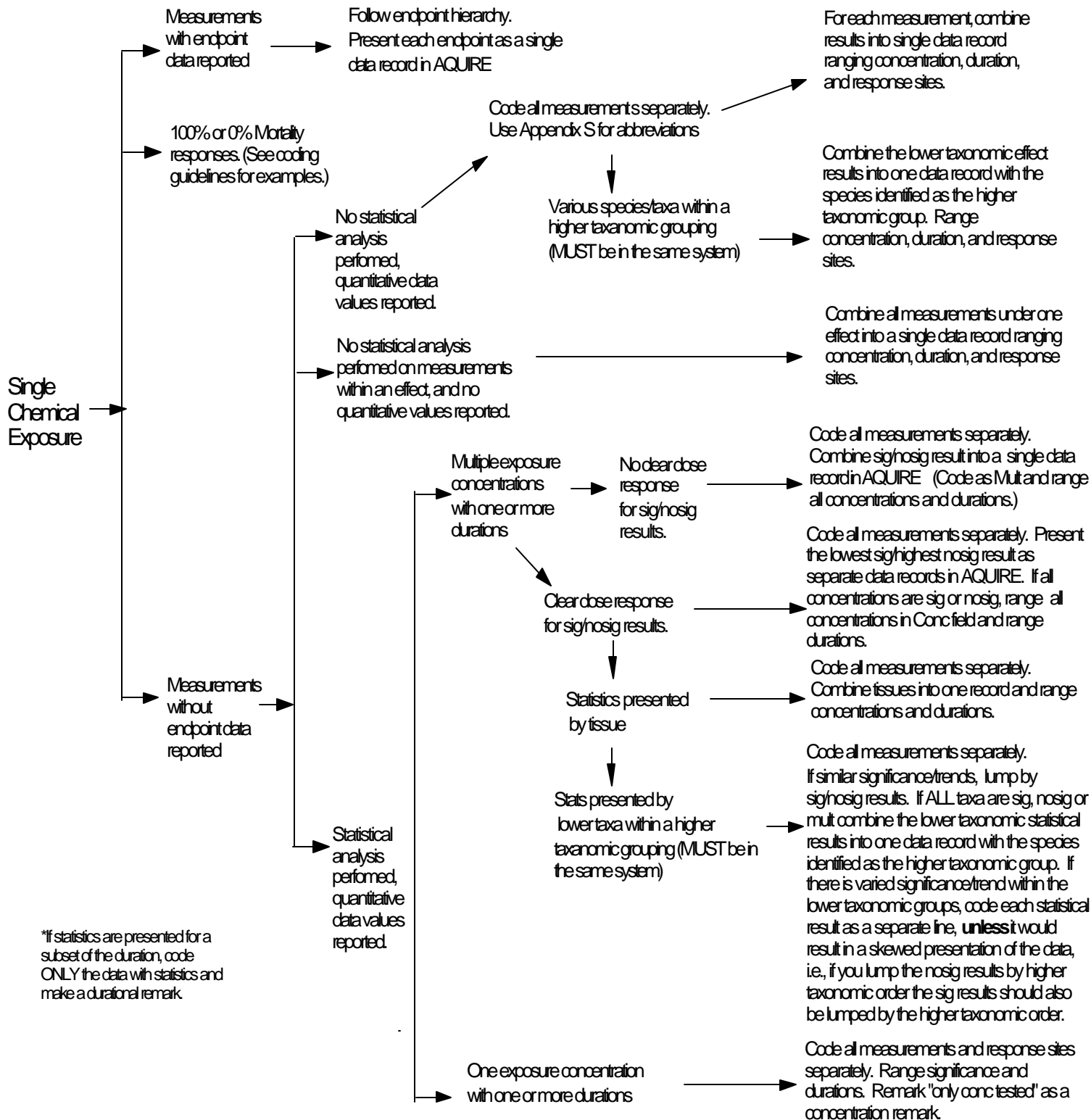
If replicate tests resulting in a number of endpoints, (e.g. LC50s), are conducted, each LC50 must be reported on an independent line, even though the chemical, species, duration and effect are the same. Mean results are not coded if individual results are reported,

Example: Rep 1 - LC50 = 23 ug/L Code only Rep 1 and Rep 2.
 Rep 2 - LC50 = 25 ug/L
 Mean - LC50 = 24 ug/L

If a data set is evaluated using more than one statistical analysis all resulting endpoints are coded on separate lines (e.g. 2 LC50s for same data using probit and Spearman-Kärber will be coded

as two separate data lines; report statistical method in EE REMARK). Additionally, note “statistical comparison” in the OTHER EFCT field (see OTHER EFCT section for more information).

Figure 1. Hierarchy of coding tests in AQUIRE



ENDPOINT HIERARCHY

The following hierarchy defines the priority for including endpoint information in the AQUIRE database. The endpoints listed in category “A” are the highest priority, based on conformance with standard toxicity endpoints, and should be coded if reported in the publication. If the endpoints identified in subsequent categories (F) are also listed in the publication, these endpoints are not coded but are noted in the OTHER EFCT field. If there are no endpoints from category “A” in the publication, then endpoints from category “B”, if available, are coded and so on.

Following the endpoint hierarchy, the next two sections define and describe the coding of trends and effects. Trend information is coded, when available for endpoints as well as effects. Regardless of whether endpoint data is available, any reported effect information is coded.

- A The endpoint is an LC50, LD50, LETC, EC50, IC50, NOEC, LOEC, MATC or BCF (BCFD), or its definition, as reported by the author. For example, if the author does not actually state that the value is an LD50 but states that “concentration x is the dose estimated to be lethal to 50% of the test organisms ” and refers to statistical methods to estimate 50% lethality, the reviewer should code this as an LD50 endpoint because the author *defines* the LD50. All individual endpoints are coded. If both BCF and BCFD (wet weight and dry weight, respectively) are presented, code only the BCFD.

The AQUIRE database recognizes and codes “companion endpoints”; for AQUIRE such endpoints are defined as statistically significant endpoints that neighbor an author-defined NOEC or LOEC.

When a publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, but note TREND as ‘CHG’ and code the SIG field as MULT to flag non-standard results.

In the AQUIRE database, the occurrence of no mortality (0%) or complete mortality (100%) is treated as an endpoint. The endpoints NR-LETH and NR-ZERO will always be coded for mortality effects of 100% mortality and 0% mortality, respectively. If for a laboratory test exposure the authors report “all fish died”, code as NR-LETH and 100% mortality; however, for a field exposure, unless conducted in an enclosure of some type, it is difficult to assume that truly 100% of the fish are known to be dead, therefore the field exposure report of “all fish died” is coded POP, DEC, ABND, and EFCT% is not coded. The term “nil” is defined as “naught or nothing”, therefore, when used by an author, it will be assumed to mean 0% mortality and coded as NR-ZERO.

The 100% mortality data point at the lowest concentration/ shortest duration is coded. Similarly, the 0% mortality data point at the highest concentration/ longest duration is coded. In contrast to other endpoints, the additional mortality effects are coded along with the NR-LETH and NR-ZERO endpoint data. For example:

Mortality Table 1

µg/L	24 H	48 H	72 H	96 H
1	0	0	0	0 NR-ZERO
2	5	17	30	35
3	25	40	65	90
4	100 NR-LETH	100	100	100

A) LC50s reported in publication, code

LC50s as reported
NR-LETH: 4 µg/L at 24 hr
NR-ZERO: 1 µg/L at 96 hr

B) LC50s not reported in publication, code

NR-LETH: 4 µg/L at 24 hr
NR-ZERO: 1 µg/L at 96 hr
MOR: 2-3 µg/L at 24-96 hr

Mortality Table 2

µg/L	24 H	48 H	72 H	96 H
1	0	0	0 NR-ZERO	11
2	20	25	38	72
3	45	60	67	90
4	90	100 NR-LETH	100	100

A) LC50s reported in publication, code

LC50's as reported
NR-LETH: 4 µg/L at 48 hr
NR-ZERO: 1 µg/L at 72 hr

B) LC50s not reported in publication, code

NR-LETH: 4 µg/L at 48 hr
NR-ZERO: 1 µg/L at 72 hr
MOR 1-4 µg/L at 24-96 hr EFCT%: 0-100

Mortality Table 3

µg/L	24 H	48 H	72 H	96 H
1	0 START RANGE	0	7	13
2	0	28	45	60
3	38	44	67	100
4	38	60	100	100 END RANGE

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR 1-4 µg/L at 24-96 hr EFCT% 0-100

Mortality Table 4

µg/L	24 H	48 H	72 H	96 H
1	0 START RANGE	0	0	0
2	0	0	7	13
3	0	28	45	60
4	38	44	67	100
5	38	60	100	100
6	100	100	100	100 END RANGE

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR 1-6 µg/l at 24-96 h, EFCT% 0-100

B The endpoint is an author reported TLM, TL50, chronic value (ChV) or any terms with equivalent definitions that define endpoints such as those listed in ECOTOX Appendix T.

The equivalent AQUIRE endpoint is coded in the ENDPOINT field.

- C The endpoint is LCxx, LDxx, ECxx, ICxx (other than 50% value). The endpoint is coded only if the endpoints listed in A or B are not abstracted from the publication.

- D The endpoint is LT50, ET50. The endpoint is coded only if the endpoints listed in A and B and C are not abstracted from the publication.

- E The endpoint is LTxx, ETxx. The endpoint is coded only if the endpoints listed in A, B, C and D are not abstracted from the publication.

- F The endpoint is a delayed exposure effect (~xxx). The delayed effect endpoint is coded if no similar exposure endpoint above has been coded. A specific exception is gut clearance prior to tissue analysis; e.g., "after the exposure the organisms were placed in clean water for 10 hours to allow the organism to clear the stomach contents". This type of clearance is distinguished from depuration and is not coded as a delayed effect.

Assigned Endpoint (ASSIGNED ENDPOINT)

If the reviewer enters an endpoint which is not stated by the author in the paper, or interprets an author's endpoint definition to be equivalent to the AQUIRE endpoint definition, a "R" for reviewer-assigned endpoint is entered into the field. However, if the reviewer codes an endpoint as the author states it in the paper, a "P" is coded for paper-assigned endpoint.

- Example 1. Author reports NOEC in paper but does not include companion endpoint - LOEC. If reviewer can determine LOEC from data, a "R" is coded for the LOEC and a "P" is coded for the NOEC.

- Example 2. Author uses TLm for 50% mortality endpoint. Reviewer can code the endpoint as LC50 and puts "R" for reviewer assigned endpoint.

Trend (TREND)

The observed or measured response trend as compared to the control is coded when reported or graphically displayed.

When assigning a trend to a record, it should reflect the measurement which may or may not reflect the effect. For example, when authors report a decrease in survival; the effect is reported as MOR, and the trend is associated with the measurement; i.e., decrease in survival.

Example: EFCT: MOR TREND: Dec MEASURE: SURV

The trend for BCF, LCxx, LTxx is coded as "inc", except for the effect SVC (shell valve closure) which is coded as "dec". The trend for ECxx, NOEC, LOEC, and MATC will be either "inc", "dec", "chg" or NR depending on the results of the test. In instances when a trend is non-monotonical

code “chg”. The trend is noted as a two or three letter code:

<u>CODE:</u>	<u>TREND:</u>
INC	increase
DEC	decrease
NEF	no observed effect; e.g., when coding NR-ZERO the trend is NEF
CHG	no clear trend, results are variable (e.g. any combination of above trends listed)
NR	no trend reported <u>or</u> if no control response is reported then the trend is not able to be identified

Example: When a clear response, or lack thereof, is observed within an effect, it is coded as either INC, DEC, or NEF. The measurement used to evaluate the effect is reported in the MEASURE field, for example:

EFCT: GRO TREND: INC MEASURE: LGTH

When measurements do not report quantifiable data (see EFFECT MEASUREMENT section), data are combined into one record. If these data report multiple trends, code CHG in the TREND field and report the individual trends in EE_REMARK field as in the following example:

Example: EFCT: HIS TREND: CHG MEASURE: GHIS
 EE_REMARK: inc EDMA, vacuolization, dec epithelial lining

Effect (EFFECT)

For ECOTOX database purposes, a toxicological effect is the observation or measurement of a response resulting from the action of a chemical stressor (e.g., mortality). The ECOTOX database internally categorizes all observed effects under at least one of eleven major effect group codes (Accumulation, Behavior, Biochemical, Cellular, Growth/Development, Lethal, Physiological, Population Community, Reproduction, Ecosystem and multiple groups). ECOTOX Appendix R describes the major groups and associated effect definitions for each three letter code. The major effect groups are not used by reviewers; their purpose is to provide database users the capability to search on broad groups of effects without specifying each individual effect. See Scientific Outreach Support for additional user support information.

The reported effect is interpreted to conform to the AQUIRE defined effects. If the effect is on the list of AQUIRE effects, use the AQUIRE effect code (see ECOTOX Appendix S). If the author's effect is not in Appendix S, but is similar to one already defined use the AQUIRE code which matches the definition and note the author's effect term in the EE_REMARK field. If the author's effect appears to be a new effect code, discuss and forward to EPA Data Manager for approval.

Listed at the end of ECOTOX Appendix R there are two special effect code conventions used in AQUIRE. The first is NOC (No effect code) used only for ENDPOINTS reported by the author as multiple effects, e.g. “mortality and growth” and no specific effect code can be assigned. This code is used *only* when such effects cannot be separated into or reported as individual effects. The NOC code is rarely used and when used must be verified by one or more fully trained reviewers.

The second effect code convention is ~XXX to indicate that the result reported was observed after the exposure period ended and the organisms are observed in clean water, i.e., a delayed response. Within a publication, delayed response data is reported only if exposure period observations are not available for the same effect or endpoint. When delayed response data accompanies exposure period observations, the delayed response data is not coded but is recorded in OTHER EFCT as “recovery”.

NOTE: A specific exception is gut clearance prior to tissue analysis; e.g., “after the exposure the organisms were placed in clean water for 10 hours to allow the organism to clear the stomach contents”. This type of clearance is distinguished from depuration and is not coded as a delayed effect.

Occasionally, effects describing a parasite-host relationship are coded in AQUIRE. For example, the effect on the host is typically coded as a PHY effect with the measurement code PRNF. The effect on the parasite is typically coded as a POP effect with the measurement code ABND.

EFFECT HIERARCHY

- A. If the author has defined an Endpoint for an effect, report the Endpoint as outlined in the preceding ENDPOINT HIERARCHY.
- B. When only effects are reported in the publication, no endpoints, code the concurrent effects (results reported concurrent with exposure to chemical) according to the abbreviations in ECOTOX Appendix S. Code NR (not reported) in the ENDPOINT field.
 - i. If statistics are presented in a clear dose response, code the lowest significant effect and the highest nosig levels and appropriate p-values.
 - ii. If statistics are presented and there is no clear dose response, code as a MULT and the appropriate value.
 - iii. If no statistics are used, or reported, combine the effect data by coding a range for concentration and duration. Report as NR in the SIG and LEVEL fields.
- C. When the only effects that are reported are those subsequent to exposure, report these as delayed effects, noted with a ~ preceding the three-letter effect code, e.g. ~MOR. Follow the procedures outlined in Steps B i, ii, iii for reporting delayed effects.

Response Site (RESP SITE)

A response site code is used to identify specific organ and tissue effect sites for residue, biochemical and/or physiological effect measurements. For example, response sites are used for ACC, BIO, CEL, HIS, PHY, GRO, and MPH effects and associated endpoints. The two or three letter response site codes are listed in ECOTOX Appendix U. It is acceptable to code a response site when tissues or organisms are pooled together for a measurement.

If data for a number of tissues are presented along with statistical results report results for each tissue separately. If statistics are not presented, combine the results into one data record.

Combining Response Site

When the residue measured in one organ or tissue is further analyzed to indicate concentrations in cells or cellular fractions, a comment is placed in the REMARK field (e.g., SITE /subcellular fraction// or SITE /subcellular distribution//).

If the MT/ code is used, the individual tissues/organs are coded in the REMARK field (e.g., Site /LI,KI,GI//). If the response site does not have a response site code, write out the response site name and include a note with the coding sheet requesting a new code be added. When the response site is not reported, the field is coded as NR. If whole organism and multiple sites are listed, code "MT/" in tissue field and code WO and additional specific tissue codes in the REMARK field (e.g. SITE/WO, LI, GI, HE//).

Effect Measurement (MEASURE)

Generally, "measures" or "measurements" are variables used to aid in the interpretation of the degree of response to a toxicant by an organism. For example, measures of behavioral effects in ECOTOX include behavioral changes (BEH SWIM), changes in feeding activity (FDB FDNG), and stimulus avoidance (AVO STIM). ECOTOX Appendix S lists the measurements currently used for each of the effects in the ECOTOX database.

Each measurement that reports quantitative data (i.e. numeric values in the text, tables, or graphs), regardless of statistical analysis, receives a separate data line. However, measurements that are discussed in the text and do not report any numeric values may be combined into one record. If several measurements are combined, code the General measurement code in the MEASURE field and list the separate measurements in the EE REMARKS field.

Example 1: Length and weight reported in table statistically analyzed.

CONC	0	10 ug/l	20 ug/l	30 ug/l	40ug/L
Length	-	nosig	nosig	sig	sig
Weight	-	nosig	sig	sig	sig
Code: EFCT: GRO		MEASURE: LGTH	CONC: 20ug/L - nosig 30ug/L - sig		
EFCT: GRO		MEASURE: WGHT	CONC: 10ug/L - nosig 20ug/L - sig		

Example 2: A decrease in length and weight reported on graph which is not statistically analyzed but values are given.

CONC (from graph)	0	10 ug/l	20 ug/l	30ug/L
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Code:	EFCT: GRO	TREND: DEC	MEASURE: LGTH	CONC: 10-30ug/L
	EFCT: GRO	TREND: DEC	MEASURE: WDTN	CONC: 10-30ug/L

Example 3: Histology reported in text. At concentrations of 10 ug/l , 20 ug/l and 40 ug/l, Reports that liver had edema, swelling, and hypertrophy.

Code:	EFCT: HIS	MEASURE: GHIS	EE remark: EDMA, SWEL, HYP
	CONC: 10-40 ug/l		

More information on coding statistics is found in the statistics section of this SOP.

Many publications which report field data or laboratory microcosm studies present results for multiple species/taxonomic groups. The combining of results for species and taxonomic groups depends on whether statistics were applied to the data and whether a similar response is evident.

- If, within a higher taxonomic group (e.g., Algae), individual effects for several lower taxonomic groups are also presented (e.g., Bacillariophyta (diatoms), Chlorophycota (green algae), Pyrrophyphyta (dinoflagellates)) the data may be reported in a number of ways. Examples include:

- The measurements within each group are statistically analyzed and are similar overall, ie., INCreasing, DECreasing or CHanGing. Combine the results and code as:

Species: Algae	ORG CHARAC: 3 orders	EFFECT: POP	MEASURE: ABND
TREND: INC	SIG: SIG		

- The measurements within each group are statistically analyzed and differ from each other. Code each result as a separate line:

Species: Bacillariophyta	EFFECT: POP	MEASURE: ABND	TREND: INC	SIG: SIG
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Species: Chlorophycota	EFFECT: POP	MEASURE: ABND	TREND: DEC	SIG: SIG
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- If no statistical analysis has been reported, the results from the lower taxonomic groups can be combined into a single record representing the next highest representative taxonomic group.

Species: Algae	ORG CHARAC: 3 orders	EFFECT: POP	MEASURE: ABND	
			TREND: CHG	SIG: NR

EE Comment (EE REMARK)

This field contains additional endpoint and/or effect text, as described by the author. The types of information coded include:

Example 1: The endpoint terminology used by the author when an ECOTOX-defined endpoint was coded

rather than the author's term. For example,

ENDPOINT: LC50 EE_REMARK: TLM or Median Period of Survival//

Example 2: If there are multiple measurements for the coded effect, all of the measurements are listed in the EE REMARK field.

EFFECT:HIS MEASURE: GHIS EE_REMARK: EDMA, LESI, epithelial lining//

If there are no remarks pertaining to either the endpoint or the effect, the field is left blank. As much as possible, codes should be used in EE_REMARK, but use of text is appropriate to ensure an understanding of the test result.

Effect % (EFCT %)

The EFCT% field is used when the effect is reported as a percent change, e.g. percent of the total population or percent increase or decrease.

If the author reports the number dead (i.e., "5 of 20") do not recalculate as a percent.

Example 1:	"80% mortality"	EFCT: MOR	TREND: INC	EFCT %: 80 MEASURE: MORT
Example 2:	"25% survival"	EFCT: MOR	TREND: DEC	EFCT%: 25 MEASURE: SURV
Example 3:	"5 of 20 died"	EFCT: MOR	TREND: INC	EFCT%: NR MEASURE: MORT
Example 4:	"45% inc ATPase activity"		EFCT: ENZ EFCT%: 45	TREND: INC Measure: ATPA

If the percent effect is coded from a graph, code the percent values using a qualifier, ie. <, >, or ~, using only the graphical intervals reported on the graph. Place a slash in the field and code EFCT%/ from graph// in the REMARK field. If the percent effect is graphed and is not clear enough to extrapolate, code "graphed" in EFCT % field. If the effect percent is not reported, the field is coded as NR.

If the percent effect is presented as "xx% of the control", place a "/" in the EFCT % field and code: EFCT %/xx% of control// in the REMARK field.

Example: GRO WGHT Dec 20-30% of the control

EFCT: GRO MEASURE: WGHT EFCT%: / TREND: DEC EE_Remark: Efct%/20-30% of control//

Combining Effect Percent

When data for an effect are combined because a statistical analysis was not applied and/or a clear

dose response was not observed, and several percent effect values are presented, there are two different ways to report data.

If the author reports the effect measurement on a single parameter, the effect percent is reported as a range.

Example 1: 30-75% mortality EFCT: MOR MEASURE: MORT TREND: INC
EFCT%: 30-75

Example 2: 20-30% dec O₂ consumption EFCT : PHY MEASURE: OXYG TREND: DEC
EFCT%: 20-30

Statistical Significance (SIG)

The statistical significance field is coded when the author has presented statistical analysis as compared to the controls in the test result.

The valid codes for this field are:

<u>CODE</u>	<u>DEFINITION</u>
SIG	Concentration(s) identified as significant (code lowest concentration in series of significant treatments)
ASIG	All toxicant concentrations significant
NOSIG	Concentrations identified as not significant (code highest concentration in series of non-significant treatments)
ANOSIG	All toxicant concentrations not significant
MULT	Combination of sig and nosig results; pattern of significance is non-monotonical, or results combined due coding practices.
NA	Not applicable (use for LC50, EC50, BCF, MATC, NR-LETH, AND NR-ZERO)
NR	Not reported

If statistics are presented in the publication, unless the authors state otherwise, assume that the exposure treatments were compared to the controls.

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), code M in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in EE REMARK. (e.g., stats based on solvent control//) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such in the EE REMARK field.

Data is separated into individual records if statistics are based on concentrations and a clear dose response is shown. However, responses reported over time are exceptions to this rule. For example:

- 1) If only one concentration is presented and the response differs over time, code one record, ranging the

durations.

Example 1:

Conc.	24h	48h	72h	96h
1 ug/l	nosig	nosig	sig	sig

Code: CONC: 1 ug/l EXP TIME: 24-96 h SIGNIF: MULT

2) If multiple concentrations are presented and the results are non-monotonic across concentrations and time, code one record combining all concentrations and all durations.

Example 2:

Conc.	24h	48h	72h	96h
1 ug/l	nosig	nosig	nosig	sig
2 ug/l	nosig	nosig	sig	sig
3 ug/l	nosig	sig	sig	sig

Code: CONC: 1-3 ug/l EXP TIME: 24-96 h SIGNIF: MULT

3) If multiple concentrations are presented and the results are monotonic across concentrations and time, code two records, a NOSIG record combining all durations and a SIG record combining all durations.

Example 3:

Conc.	24h	48h	72h	96h
1 ug/l	nosig	nosig	nosig	nosig
2 ug/l	sig	sig	sig	sig
3 ug/l	sig	sig	sig	sig

Code: CONC: 1 ug/l EXP TIME: 24-96 h SIGNIF: NOSIG

Code: CONC: 2 ug/l EXP TIME: 24-96 h SIGNIF: SIG

The SIG field is coded as "NA" for records having an endpoint of MATC, LCxx, ECxx, LTxx, BCF, ETxx, ICxx, LDxx, LETC, BCFD, NR-LETH, and NR-ZERO. For NOEC, LOEC and effects without endpoints, code significance as author reports, or NR.

When a publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, but note TREND as 'CHG' and code SIG as 'MULT' to flag non-standard results.

The reviewer interprets hypotheses tests to determine a dose response endpoint. A significant clear dose result is coded as SIG; no significant dose result is coded as NOSIG. Only the highest NOSIG and the lowest SIG concentration is reported; unless all concentrations are SIG or all concentrations are NOSIG. In this instance, code all the concentrations as a range as ASIG (all significant) or ANOSIG (all not significant) respectively. If the significance level is reported, it is coded in the LEVEL field described below.

In cases where the author reports only a SIG or NOSIG, code the companion data point. For example, if a stat sig "growth" is reported in the text and in the table sig is noted the reviewer should pick the nosig level and report this also.

If the author states that there is a statistically significant increase or decrease in an observed effect, whether or not they report the statistical method used, but does not report a significance level, code SIG or NOSIG and NR in LEVEL field.

If a table has a footnote defining * values as significant as $p < .05$, it is acceptable for the reviewer to assume that data points without an asterisk are not significant.

If the author states there is a significant increase or decrease in an observed effect but does not say it is "statistically significant," code NR in SIG field.

- When the highest concentration and all lower concentrations tested show no significant response, code ANOSIG and range all concentrations in CONC field.

Example:

10 ug/L	NOSIG	P<0.05
20 ug/L	NOSIG	P<0.05
30 ug/L	NOSIG	P<0.05

Code: CONC: 10-30 ug/L SIG: ANOSIG LEVEL: P<0.05

- When the lowest concentration and all higher concentrations tested show a significant response, code ASIG and range all concentrations in CONC field.

Example:

10 ug/L	SIG	P<0.05
20 ug/L	SIG	P<0.05
30 ug/L	SIG	P<0.05

Code: CONC: 10-30 ug/L SIG: ASIG LEVEL: P<0.05

- If only one concentration is tested and statistics are performed, code SIG or NOSIG in stats and "only conc tested" as a CONC remark.

Example:

10 ug/L	SIG	P<0.05
---------	-----	--------

Code: CONC: 10ug/L SIG P<0.05 REMARK: Conc/only conc tested//

Combining of Statistics

If a measurement has no clear dose response as interpreted by the reviewer when statistics are reported, it is coded as multiple significance (MULT).

- Example 1: Five concentrations are tested and the two highest and two lowest show significance but the middle concentration does not, code MULT.
Growth table with length affected by copper.

Conc	Control	1 ug/L	2 ug/L	3 ug/L	4 ug/L	5ug/L	
Length	20 cm	35cm	36cm	21cm	15cm	14cm	
		sig	sig	nosig	sig	sig	P<0.05

Code: EFCT: GRO MEASURE: LGTH SIGNIF: MULT LEVEL: p<0.05
CONC: 1-5 ug/L

- Example 2: Change in calcium concentration in the blood over three sample times shows significance at 24

hours, no significance at 48 hours, and significance at 96 hours. Code SIG field as MULT.

[Calcium]	24 hours	48 hours	96 hours	
Control				
10 ug/L	sig	nosig	sig	P<0.05

Code: EFCT: BCM MEASURE: CACO SIGNIF: MULT LEVEL: p<0.05
 CONC: 10 ug/L DURATION: 24-96 H

Example 3: A publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, note TREND as 'CHG', and code SIG as 'MULT' to flag non-standard results.

Mortality	Control	10 ug/L	20 ug/L	30 ug/L	40 ug/L	50 ug/L
@ 24 hours		nosig	nosig	sig	nosig	sig
Author endpoints			NOEC	LOEC		

Code: ENDPT: NOEC EFCT: MOR MEASURE: MORT CONC: 20 ug/L
 SIGNIF: MULT TREND: CHG

ENDPT: LOEC EFCT: MOR MEASURE: MORT CONC: 30 ug/L
 SIGNIF: MULT TREND: CHG

Note: The MULT code is not used to represent a combination of data which has been statistically analyzed with data that has not been analyzed. Code the statistical data over the non-statistically analyzed data. For example, in an instance where the exposure duration is 5 days, and the statistical results presented are for 4 days; code the statistical results, note the duration as 4 days., place a slash in the duration field, and enter the exposure duration (5 days) in the Remark field.

Significance Level (LEVEL)

The level of significance (e.g. test statistic) is coded when the author has reported statistical analysis in the test result. The terminology for significance level may be presented as: p =; p- or alpha value; χ^2 ; for t-test; % level. The terminology are equivalent and are generally in the range of p = 0.10 to p = 0.001.

The LEVEL field is coded as "NA" for records having an endpoint of MATC, LCxx, ECxx, LTxx, BCF, ETxx, ICxx, LDxx, LETC, BCFD, NR-LETH, and NR-ZERO. However, when the confidence level is other than 95%, the level is coded as reported.

Combining of Level

When a range of concentrations is coded, and there are multiple levels of significance reported, range the values.

Example: At all concentrations (10-50 ug/L) growth was significantly affected. At 10 ug/L the p value was p<0.05, at 50 ug/L the p value was p<0.001.

CONC: 10-50 SIG: SIG LEVEL: P<0.05 - 0.001

Other Effects (OTHER EFCT)

Comments regarding other toxicity tests or effects reported in the publication that do not meet AQUIRE minimum data requirements are coded in this field. A keyword list (see ECOTOX Appendix V) for common terms is used as a guideline to assist the reviewer. The effect or endpoint codes are used when appropriate. The reviewer should maintain a list of new keywords and periodically submit this list to the EPA Database Manager. Commas separate each distinct term and the text ends with a double slash (/).

OTHER EFCT: uptake, elimination//
 OTHER EFCT: toxicity symptoms, diet study//
 OTHER EFCT: mixture, effluent//

If other chemicals are tested as a mixture with the test chemical, the keyword "mixture" is coded in the OTHER EFCT field.

When water chemistry effects (temperature, salinity, pH) are tested in conjunction with chemical toxicity, a Remark is coded in OTHER EFCT to reflect this type of interaction.

OTHER EFCT: salinity efcts//

Test Result Examples

1. If the author has defined an ENDPOINT and/or has reported a 0% and/or 100% mortality response, report the endpoint/mortality as outlined in the Endpoint Hierarchy. Select the appropriate effect as described below.

ENDPOINT REPORTED (NR-ZERO):

ENDPOINT: NR-ZERO	MEASURE: MORT	
TREND: NEF	EFCT%: 0	
EFFECT: MOR	SIG: NA	LEVEL: NA

If applicable, statistical results should appear in the SIG field, the level of significance should be reported in the LEVEL field, the percent effect should be presented in the EFCT% field, and the trend should be reported in the TREND field.

ENDPOINT REPORTED (LOEC):

ENDPOINT: LOEC	MEASURE: LGTH	
TREND: DEC	EFCT%: 20	
EFFECT: GRO	SIG: SIG	LEVEL: $\alpha < 0.05$

Note: For NOEC endpoints, NOSIG is coded in the SIG field. For LOEC endpoints, SIG is coded in the SIG field.

2. If the author-reported effect is a clear dose response result using statistical analysis, and the author does not identify an endpoint, select the appropriate effect from ECOTOX Appendix S.

Clear dose response data where a statistically significant effect was observed, are represented by two data records. One data record represents the lowest concentration at which a statistically significant effect occurred. "SIG" is coded in the SIG field, the observed trend is coded in the TREND field, the percent effect is coded in the EFCT% field, and the level of significance is reported in the LEVEL field. The observed measurement is coded in the MEASURE field. Remarks on the effect are made in the EE_REMARK field.

CLEAR DOSE RESPONSE:

ENDPOINT: NR	SIGNIF: SIG	TREND: DEC
EFFECT: GRO	EFCT%: 20	LEVEL: $\alpha < 0.05$
MEASURE: LGTH		

The second data record represents the highest concentration at which no effect occurred. NOSIG is coded in the SIG field. If a percent effect is reported it is presented in the EFCT% field.

If the concentration identified as SIG is the lowest concentration reported or the concentration identified as NOSIG is the highest concentration reported, report the range of concentrations and the appropriate code (ASIG and ANOSIG) in the SIG field.

If only one concentration is tested, code the SIG field appropriately and note "only conc tested" as the concentration (CONC) remark in the REMARK field.

3. If the author reported effect shows unclear dose response results, using statistical analysis, select the appropriate effect from ECOTOX Appendix S.

When data have been statistically analyzed, and the results presented have significant effects in an unclear dose response pattern (e.g., significant effects at the high and low concentrations, and not significant at the middle concentration), "MULT" is coded in the SIG field to signify multiple significance. The level is coded with a full range of p-values (e.g. $p < 0.05$ -0.001).

UNCLEAR DOSE RESPONSE:

ENDPOINT: NR	MEASURE: ACHE	
TREND: CHG		
EFFECT: ENZ	SIG: MULT	LEVEL: $P < 0.05$ -0.001

4. If the author reports a descriptive or qualitative effect without statistical analysis, select the most appropriate effect from ECOTOX Appendix S. One record is coded with a full range of exposure concentration and time. The appropriate trend is coded in the TREND field. The percent effect over the concentration tested is reported in the EFCT% field. NR is coded in SIG and LEVEL fields.

NO STATISTICAL ANALYSIS:

ENDPOINT: NR

MEASURE: GHIS

EE REMARK: LESI,
ATRS, DEGN, EDMA

TREND: INC

EFCT%: NR

EFFECT: HIS

SIG: NR

LEVEL: NR

6. Concentration Parameters

Concentration Type (CONC TYPE)

The three forms of toxicants evaluated in AQUIRE are organic compounds, metals and inorganic non-metals. Each form can be identified as a concentration type code using the single letter abbreviation.

Organic compounds are defined by the pesticidal terms, formulation (F) and active ingredient (A). Publications that do not specify the compound by the definition criteria for active ingredients are by default coded in the formulation (F) category.

Metals are defined by the concentration types, total (T), dissolved (D), and labile/free (L); while ammonia or hydrogen sulfide compounds may have total concentrations (T) and/or un-ionized (U) concentrations. Organometals are coded as total (T) concentrations.

If two representative concentrations of a metal or inorganic non-metal are reported in the reference, both concentrations are included in the same AQUIRE record; i.e., both total and un-ionized concentrations are reported in the concentration field. If the author reports the ammonia concentrations as based on $\text{NH}_4\text{-N}$ or $\text{NH}_3\text{-N}$, code CONC TYPE as "T" and "U", respectively in the same record and code the specific ion information in the ION fields.

For publications where all three metal types, T, D and L, are reported code T and D as one entry and the L concentration is coded as a separate line. (At some future point when new software is developed, all three concentration types will be associated with one record).

Concentration Type is also linked to the Chemical Analysis Method (METHOD) field.(see discussion below on Active Ingredient).

Concentration Types and Definitions

Organic:

FORMULATION (F): Way in which basic pesticide (toxicant) is prepared for practical use (Ware, 1978). Generally reserved for commercial preparation prior to actual use and does not include the final dilution (Insect-Pest Management and Control, 1971) (e.g.; Baythroid, 2,4-D). Also included in this category are organic compounds with no pesticidal activity (e.g.; PCB, dioxin).

ACTIVE INGREDIENT (A): Chemical substance in a product that is responsible for the pesticidal (toxic) effect (Ware, 1978). Reported as "A" when the author refers to the concentration as active ingredient, active principle or various grades of reagents (ie., Analytical, Reagent or Technical). When coding, a value in the publication may be reported as "AI kg/ha" or "kg AI/ha"; in AQUIRE this type of value is reported as 'A =' for CONC TYPE, with units

as kg/ha. For example, 100 kg AI/ha is reported as A = 100 kg/ha.

Note: Information reported in the PURITY field does not necessarily determine whether concentration is A or F. In addition to the description above, using "A" as the concentration type occurs in situations such as the following:

- 1) Author states concentration of pesticide as "AI".
- 2) Author states %AI (PURITY) and reports measured concentration.
- 3) Author states measured concentration of a pesticide.

Metal/Organometals:

TOTAL (T): The concentration of metals determined on an unfiltered sample after vigorous digestion, or the sum of the concentrations of metals in both dissolved and suspended fractions (APHA et.al. 1992). Heavy metals and single elements (e.g. Na, Cl, Br) are coded as T.

DISSOLVED (D): Those constituents of an unacidified sample that pass through a 0.45 um membrane filter (e.g. soluble metal) (APHA et.al. 1992).

LABILE (L): The labile or free ion metal concentration determined by various analytical methods. When coding, the specific labile forms or complexes are not differentiated.

Inorganic non-metals:

Concentrations of ammonia and hydrogen sulfide are reported in the literature in either the total or unionized form. Code the form as specified by the author. Ammonia may be reported as a variety of different forms, eg., NH_3 , NH_4^+ , $\text{NH}_3\text{-N}$, NH_4OH , or NH_4Cl . (US EPA 1979) The author must state whether the form is **Total** or **Unionized**; **T** is the default for ammonia and hydrogen sulfide papers that do not state whether total or unionized concentrations are reported.

TOTAL (T): The dissociated, charged form of nitrogen or hydrogen related chemicals. This can take on numerous forms, e.g.; ammonium (NH_4^+), nitrite (NO_2^-), etc. (Rand and Petrocelli, 1985). T is the default for publications that do not state whether Total or Unionized concentrations are reported.

UN-IONIZED (U): The undissociated, uncharged form of ammonia or hydrogen sulfide. The ammonia molecule, NH_3 , is the unionized form. (In aqueous solution, ammonia assumes an equilibrium between NH_3 and NH_4^+ .) The NH_3 is the toxic entity of the ammonia compound (Rand and Petrocelli, 1985).

Ionic Fraction (ION)

For ionizing substances (e.g., metals, ammonia), report the dose as the ion if the concentration presented by the authors is reported as based on the ionic form of the compound (eg., organotin as Sn). Code the appropriate ionic symbol in the ION field (see ECOTOX Appendix O for Ion codes). If concentration is based on the total compound, code 'NR' in this field. For non-ionizing substances, code 'NR' in this field.

If the test chemical for a metal is reported as the elemental metal (i.e. mercury) code the ion (Hg) in the ION field.

Effect Concentration (CONC)

Report the effect concentration in the same units used by the author. Do not convert any units. (See ECOTOX Appendix N for a list of concentration units). The confidence interval, fiducial limits, or range is recorded when available. The water concentration is coded in this field, except for diet studies, where the concentration in the food is coded. If a test is run with two sources of chemical,

Code the mean and range of a stated concentration unless there are multiple test results being combined into one test record. In this case the lowest minimum concentration and the highest maximum concentration will be used.

Occasionally an author will report a concentration as a % or fraction of an LC50 value; e.g., either the sublethal concentration used was "10% of the 96-h LC50" or "1/10, 1/15 and 1/20 of the LC50". Such concentrations may be recalculated and used as the effect concentration if the original LC50 concentration is provided in the publication. Flag the recalculation in the paper so that the calculation may be QA'd and document the recalculation in the margin or on a blank page of the publication. Put a slash next to the concentration value and note in the REMARK field:
CONC/Recalculated//.

All reported concentrations are coded and identified as to whether the concentration is based on the active ingredient or formulation, or as the total, un-ionized or dissolved concentration, are identified (see CONC TYPE).

In certain cases, the AQUIRE concentration is routinely reported as some form of the test chemical. For metal salts, the concentration is generally expressed as ug ion/L (e.g., Hg⁺). Be sure to code the ION field with appropriate ion.

An exponential number greater than +8 or smaller than -7 (e.g., 1×10^8 ; often reported as 10^8) is coded as E+n or E-n (e.g., 1 E+8). The concentration field is 10 characters long, therefore numbers

less than or equal to +8 or -7 can be written out, eg. 10^6 is reported as 1,000,000.

When the concentration is reported as the metal (e.g., Sn), but the chemical tested is identified as an organometallic (tributyltin chloride ($C_{12}H_{27}ClSn$)): Record the full name of the chemical tested in the TEST field, enter "T" in the CONC TYPE, report the concentration in the CONC field, and identify in the ion that the concentration is based on the metal component and report in the ION field.

If a chemical concentration is reported in the control water, 'contaminated controls' should be noted in the EXP DESIGN field. The concentration of chemical in the controls is not coded.

If the chemical used is an aged solution and the author is comparing the data to varying aged solutions (e.g. 2 d aged solution, 4 d aged solution and 6 d aged solution), all data are coded, "X d aged solution" is coded in the EXP DESIGN field, and "Aged Solution Efcts" is coded in the OTHER EFCT field.

For field data, the water concentration may be reported as NR, if the application rate is reported (see AP RATE field). However, the concentration type (F,A,T,D,L,U) must still be coded in this field along with NR.

Bioconcentration Value (BCF)

The bioconcentration factor (BCF) is a unitless value describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment. At apparent equilibrium during the uptake phase of a bioconcentration test, the BCF is the concentration of a chemical in one or more tissues of the aquatic organism divided by the average exposure concentration in the water. The unitless number is calculated by dividing the concentration of the exposure chemical found in the tissue by the concentration of the chemical found in the exposure water,

$$BCF = \frac{\text{g/kg chemical in organism tissue}}{\text{g/L chemical in } H_2O}$$

or it is calculated from a ratio of rate constants, if at steady state,

$$BCF = \frac{K1 \text{ (uptake)}}{K2 \text{ (elimination)}}$$

A bioconcentration endpoint is coded as either wet (or unknown) or as dry weight (BCF and BCFD, respectively). An accumulation (ACC) effect, measurement of RSDE, and the associated BCF value is coded in the BCF field. If the author does not calculate a BCF, the test is recorded as an ACC effect, measurement of RSDE, NR in the ENDPOINT field, and NR in the BCF field.

If a BCF is reported for the parent compound and for a metabolite, record the parent compound BCF and note /metabolite BCF// in OTHER EFCT.

If the BCF is at steady state or equilibrium, it is noted using the term "steady state" in the

EE_REMARK field.

If the BCF is normalized for lipid, "lipid normalized" and the % lipid, if available, are reported in the EE_REMARK field.

EE_REMARK: Steady State, lipid normalized 5% lipid//

If an author reports more than one type of BCF, ie. lipid normalized, regular, or radioactive equivalents, for the same data point; code lipid normalized over regular and regular over radioactive equivalents. The secondary analysis endpoint is reported in OTHER EFCT.

OTHER EFCT: radioactive equivalent BCF//

For papers that report BCFs and provide Lethal Body Burden information, note "Lethal Body Burden" in OTHER EFCT. However, in a publication reporting only residue data as lethal body burdens code the effect as ACC, the measurement as RSDE, and report "Lethal Body Burden" in EE_REMARK.

If an author indirectly measures the uptake of a chemical in an organism by measuring the loss of the chemical from the test media, the data is not coded. However, if the chemical is measured in the excrement (urine or feces) it is coded as RSDE in urine (UR) or feces (FC).

Exposure Type (EXP TYP)

Exposures must either be aqueous, through the diet, or by injection. Specific exposure types are coded. For example, if an injection exposure type is reported, code the specific route of injection (such as IM for intramuscular). *In vitro* toxicity test results are not coded in the AQUIRE database. If an exposure type is not clearly defined or is not reported, an NR exposure type is coded. Exposure Type codes are listed in ECOTOX Appendix J.

Chemical Analysis Method (M/U)

This parameter identifies whether quantitative analyses of the toxicant concentration in the test water was conducted and whether measured concentrations were used to report the results. This field represents/defines the concentration which was used in reporting the endpoint or effect; publications may report **M**easured and **U**nmeasured concentrations for one test scenario, use the code which represents whether the specific effect/endpoint concentration was measured or unmeasured. If both measured and unmeasured concentrations for the specific effect/endpoint are reported, record only the measured concentrations. When chemical measurements are conducted on stock solutions, but nominal concentrations are reported for effects or endpoints, code as **U**nmeasured. When chemical measurements are conducted periodically throughout the exposure but the reported measurements are not correlated with the effects, code as **U**nmeasured. When chemical measurements are conducted periodically throughout the exposure and the effects are coordinated with the measurements, code as **M**easured. For non-English publications, code as **N**ot **R**eported unless explicitly stated to be measured or unmeasured concentrations.

Even if measured values are reported by the author to have deteriorated by the end of the

exposure, the **Measured** code should still be used. It is acceptable to assume that if the author used measured concentrations in residue analysis, that these measured values were carried over to calculate BCF's. (See ECOTOX Appendix P for codes)

7. Test Duration Parameters

Exposure Duration (EXP TIME)

Exposure duration is coded using the units reported in the publication. If exposure duration is not reported, the publication is rejected (unless it is an abstract or is a non-English publication). Time information may be extracted from a figure.

For a fluctuating or intermittent dosing (P) experiment, the total test time is recorded in the EXP TIME field with the exposure times and intervals between dosages reported in the REMARK field.

Example: EXP TIME: 24 Duration Unit: H/ Remark: TIME/ 3 pulses of 45 mi each per 24 H//

When an exposure duration is not directly linked to a response, the duration is reported as the full range of time, e.g. "during a 10 week period" is coded as "0-10 wk" or if the response is for a portion of the exposure duration, ie., from day 2 through 10 wk, then code as 2-70 d.

For delayed effects, report the duration of exposure to the toxicant only. The observation time is not recorded. (See ECOTOX Appendix I for valid duration units)

For generational studies, report results only for lifestages that are directly exposed to the toxicant.

Example 1: Parent exposed – Offspring in clean water.
Code effects on parents and code "generational study" in OTHER EFCT.

Example 2: Parents exposed – Offspring exposed
Code the effects on both parents and offspring. Code the initial lifestage of organisms tested in the ORGANISM CHARACTERISTICS field and code the lifestage of the organism being measured in the EE REMARK field.

8. Water Chemistry Parameters

The following water chemistry parameters are included in AQUIRE, and are coded in appropriate fields. These measured values pertain either to the test water chemistry or the dilution or culture water chemistry values. In the absence of test water chemistry parameters, it is acceptable to report the culture, holding tank, acclimation, control or dilution water, or pretest conditions denoted by an asterisk (*). Water chemistry parameters measured prior to or after the exposure period are coded only if test water chemistries are not reported in the publication. If the author reports the test conditions as "similar" to other methods in the paper, code test conditions as "NR".

When water chemistries differ between samples (e.g., test chamber or water body), and results are obtained from only some of the samples, water chemistries should be reported for only those samples actually tested.

If the parameter unit is any unit other than the standard unit code (see Table 2) the unit is coded with the value. When the author refers to the water chemistry values as approximate a "~" is coded in front of the value. Graphed data are coded as a range or as "less than" or "greater than" values and the term "graphed" is noted as a REMARK, e.g. temp/graphed//.

Water chemistry values should be coded as reported by the author. If the author uses the standardized units of the AQUIRE, the units do not need to be recorded. (See ECOTOX Appendix W for additional water chemistry units)

Table 2. Specific Parameters

Field Name	Standard Unit	Definition	Comment
<u>TEMP</u>	C	Temperature	When temperatures are reported for incubation chambers or water baths, these temperatures are acceptable for reporting as test temperatures. Do not code temperatures noted as "room temperature".
<u>HARD</u>	mg / L CaCO ₃	Hardness	If the author only reports the terms "hard" or "soft", these terms are recorded. If the author reports a hardness value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded.
<u>ALK</u>	mg / L CaCO ₃	Alkalinity	If the author reports an alkalinity value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded.
<u>DO</u>	mg / L	Dissolved Oxygen	A "SAT" code is used for 100% saturation
<u>pH</u>	--	pH	pH range is between 1 and 14
<u>SALIN</u>	ppt	Salinity	
<u>COND</u>	umhos / cm	Conductivity	
<u>ORG C</u>	mg / L Carbon	Organic Carbon	Organic carbon must designate (T=Total, P=Particulate, D=Dissolved); if more than one type of organic carbon is reported in the publication, record T in the field and the other values (P or D) as a Remark; if the value is reported as "organic carbon" without identifying type, assume the value is expressed as Total and report T. Sediment organic carbon values are not reported.

9. Remark Parameters

The REMARK field contains additional information about a coding field. The coding sheet does not reflect a discreet REMARK field. Reviewers should code remarks in available blank space. Remarks for an AQUIRE field begin with a field name identifier, then a slash (/), followed by text and end with a double slash (/).

Example: Site/LI, KI, MU //

When additional information is necessary for coding a field, a slash is placed in the coded field and a remark field name identifier is placed in the REMARK field to link the remark to the coded field. A complete list of field names is documented in ECOTOX Appendix DD.

10. Field Testing Parameters

Habitat Comment (HABITAT CODE)

In the first box, a one-letter code based on the Cowardin system code (see ECOTOX Appendix X) is used to describe the habitat (eg., **L**acustrine or **R**iverine). The descriptor field is used to record the author's description of the water body, e.g. brackish marsh, oligotrophic lake, plastic tub, polyethylene lined enclosure. If the author does not provide any information about the habitat, both fields are coded as NR (not reported).

Habitat Code	
P	concrete tanks in natural pond

Substrate (SUBSTRATE)

The bottom substrate is recorded as a two letter code by using the SUBSTRATE codes listed in ECOTOX Appendix Y. If there are no applicable codes, record as the author states in the literature. If a substrate is not reported, NR is recorded. A mixture of sediment types is coded as "MX" and should also include text for the most prevalent soil type(s) in the mixture.

Differentiate between organic and mineral soil/sediment by recording O for organic (leaves, detritus, debris) and M for mineral. Report % organic matter, if reported in literature.

Substrate	
MX	SA, GR, rocks

Water Depth (DEPTH)

Water depth value and unit are coded for the study site, as reported by the author. The software will convert the depth to a metric unit. "NR" is coded in the DEPTH field if the author does not report the water depth at the study site. If the author only reports the water depth of the entire system or the depth at which experimental units (i.e., cages) are suspended, "NR" is coded, and depth information is included in the EXP DESIGN field. (See ECOTOX Appendix Z for valid unit codes)

Geographic Location (LOCATION)

Water body, city, county or relevant site information is coded. (see ECOTOX Appendix AA for field location abbreviations.)

Geographic Code (ST/PR/COUNTRY)

This field will contain the state, province or country name of the test site along with the Geo code. If the test site is not reported, an "NR" is coded. (ECOTOX Appendix BB contains a listing of country, region, province names and associated Geo code.)

Longitude/Latitude (LAT/LONG)

If reported by the author the latitude and longitude are recorded. If a range is reported, place a slash in the field and report range in REMARK field. If not reported, NR is recorded. An example of a longitude/latitude location (MED, Duluth, MN) is listed below:

Latitude:	46°50'51" N
Longitude:	92°11'12" W

NOTE: The "~" sign replaces the "°" sign in data entry.

Application Type (AP TYPE)

This code will contain the method of application of the chemical. Application type codes are located in ECOTOX Appendix J.7.

For instances where the reviewer is unsure whether the chemical was applied directly to the water body by pumping, pouring, metering, etc., "DA" (Direct Application) will be coded.

Application Frequency (FREQUENCY)

Record the number of doses applied during the exposure. If the dose is a non-pulsed, continuous flow, code "continual" in the FREQUENCY field. Examples of continual exposures include artificial stream experimental systems and in situ exposures. If an application frequency is not reported, record NR. "Times" is written as X (e.g. 1X, 2X).

Examples: FREQUENCY: 3X per mo// FREQUENCY: 4X// FREQUENCY: Continual//

Application Rate (AP RATE)

This field contains the application rate value and the units that the author reports. If an application rate is not reported by the author, record as NR. If an exposure concentration is not reported by author, the application rate must be reported. (See ECOTOX Appendix CC for application units)

Chemical Half-life (HALF-LIFE)

Record the specific chemical half-life in water as measured by the author. If the half-life is

referenced from another paper, code this field as NR. If information about the half-life is not reported, record NR.

Example: HALF-LIFE: 2d

Application Season (AP SEASON)

This field is used ONLY if no application date is given by the author but the author does specify a season. This field contains the season of initial application of the chemical. A list of application seasons with dates and AQUIRE codes is presented below:

<u>Code</u>	<u>Season</u>
WI	Jan-March
SP	April-June
SU	July-Sept
AU	Oct-Dec
NR	Not Reported

Application Date (AP DATE)

The application date is the time of initial exposure. The format is MO-DA-YR, e.g. 12-01-93, 01-00-75, 00-00-64. If more than one initial date is reported (e.g. more than one pond exposed), record the dates as a REMARK. If one pond is exposed multiple times, only report the first application date and note #x in frequency. If the application date is not reported, NR is recorded.

11. References

American Public Health Association, American Waste Works Association, and Water Pollution Control Federation. 1992. *Standard Methods for the Examination of Water and Waste Water*, Eighteenth Edition, APHA, Washington, DC.

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Insect- Pest Management and Control. 1971. Vol. 3, National Academy of Sciences: 377.

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G

Replicate
Review
Complete

ECOTOX 10% REPLICATE REVIEW

Date: _____

Reference Number: _____

Section I

Coordinator Comments (Data abstraction discrepancies outlined):

Initials: _____ Date: _____

Section II

Second Data Abstractor Comments (Response to discrepancies):

Initials: _____ Date: _____

Section III

First Data Abstractor Comments (Response/Incorporation of modifications):

Initials: _____ Date: _____

Section IV (If non-applicable, go to Section VI)

Coordinator Comments (Remaining data abstraction discrepancies outlined):

[] Further action required, see below:

Initials: _____ Date: _____

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**Section V** *(If Section IV is used, complete Section V)*

First Data Abstractor Comments (Response/Incorporated modifications outlined):

Initials: \_\_\_\_\_ Date: \_\_\_\_\_

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Section VI

[] Replicate review complete; no further action required.

Initials: _____ Date: _____

	CHEMICAL	GRADE	PURITY	FORM	CHARACTERISTICS	RADIO LABEL	CAS NUMBER
5. TEST							
2. S/V							
3. S/V							

STUDY TYPE _____ QA DATE ____ / ____ / ____ INITIALS ____

EXP. DESIGN:	EE_REMARK:
OTHER EFCT:	

EXP. DESIGN:	EE_REMARK:
OTHER EFCT:	

EXP. DESIGN:	EE_REMARK:
OTHER EFCT:	

Loc No	Latin Name Species Number	Organism Characteristics	C n t	Endpoint Trend Effect Resp Site	Measure Efct % Sig Level	Conc Type, Concentration and Range or Cl ION	BCF	Exp Time	Exp Typ	M/U	Temp	Hard	Alk	D.O.	pH	Sa- linity	Cond	Org C
																		DTP
EXP. DESIGN:						EE_REMARK:												
OTHER EFCT:																		
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ACQUIRE FIELD CODING SHEET (September , 2000)																	
1. TEST		CHEMICAL	GRADE	PURITY	FORM	CHARACTERISTICS		RADIO LAB		CAS NUMBER							
2. S/V																	
3. S/V																	

REF #, AUTH FIELD		HOR, YEAR			TOTAL TESTS		REVIEWER		DATE / /		QA Date		Initials		FW SW NR			
Study Type																		
Loc No	Latin Name Species Number	Organism Characteristics	C n t l	Endpoint Trend Effect Resp	Measure Effect % Sig Level	Conc Type Concentration and Range or CI	BCF	Expo Time	E x T y	Meth-od	Temp	Hard	Alk	D.O.	pH	Sa-linity	Cond	Org C D T P

EXP. DESIGN:									EE_REMARK:								
OTHER EFCT:																	

Habitat Code		Substrate		Depth		Location:					APTyp		Frequency		AP Rate		Half Life		AP Date		AP Season	
						St/Pr/Country:																
LAT																						
LONG																						
	Name	Char	C			Conc		BCF	Time	E x	Meth	Temp	Hard	Alk	DO	pH	Sal	Cond	Org C D T P			

EXP. DESIGN:									EE_REMARK:								
OTHER EFCT:																	

	Name	Char	C			Conc		BCF	Time	E x	Meth	Temp	Hard	Alk	DO	pH	Sal	Cond	Org C D T P
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EXP. DESIGN:

EE_REMARK:

OTHER EFCT:

EXP. DESIGN:

EE_REMARK:

OTHER EFCT: